Calibration Protocols

1. OD600 Reference point

- Add 100 µl LUDOX into wells A1, B1, C1, D1 (or 1 mL LUDOX into cuvette)
- Add 100 µl of H_2O into wells A2, B2, C2, D2 (or 1 mL H_2O into cuvette)
- Measure absorbance 600 nm of all samples in all standard measurement modes in
- instrument
- Record the data in the table below or in your notebook
- Import data into Excel (**OD600 reference point tab**) Sheet_1 provided

2. FITC fluorescence standard curve

2.1 Prepare the FITC stock solution:

- Spin down FITC stock tube to make sure pellet is at the bottom of tube
- Prepare 10x FITC stock solution by resuspending FITC in 1 mL of 1xPBS
- Incubate the solution at 42°C for 4 hours
- Dilute the 10x FITC stock solution in half with 1xPBS to make a 5x FITC solution and

resulting concentration of FITC stock solution 2.5 µM.

2.2 **Prepare the serial dilutions of FITC:**

- Add 100 µl of PBS into wells A2, B2, C2, D2….A12, B12, C12, D12
- Add 200 µl of FITC 5x stock solution into A1, B1, C1, D1
- Transfer 100 µl of FITC stock solution from A1 into A2.
- Mix A2 by pipetting up and down 3x and transfer 100 µl into A3
- Mix A3 by pipetting up and down 3x and transfer 100 µl into A4
- Mix A4 by pipetting up and down 3x and transfer 100 µl into A5
- Mix A5 by pipetting up and down 3x and transfer 100 µl into A6
- Mix A6 by pipetting up and down 3x and transfer 100 µl into A7
- Mix A7 by pipetting up and down 3x and transfer 100 µl into A8
- Mix A8 by pipetting up and down 3x and transfer 100 µl into A9

- Mix A9 by pipetting up and down 3x and transfer 100 µl into A10
- Mix A10 by pipetting up and down 3x and transfer 100 µl into A11
- Mix A11 by pipetting up and down 3x and transfer 100 µl into **liquid waste**
- Repeat dilution series for rows B, C, D
- Measure fluorescence of all samples in all standard measurement modes in instrument
- Record the data in the notebook

Import data into Excel (**FITC standard curve tab**) Sheet_1 provided